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IN VITRO ADVENTITOUS SHOOT REGENERATION OF WATER HYSSOP (*Bacopa monnıerı* L. PENNEL) UNDER LIGHT EMITTING DIODES (LEDS)

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Water hyssop or Brahmi (*Bacopa monnieri*) is an important medicinal plant of ancient times and still in use due to highly effective bioactive compound knowna as Bacoside. The plant is mostly wild but is cultivated in some areas due to its demand. However, the demand is higher than its production and reserachers are continuously developing new efficint protocol by employing variable factors to meet its demand. In this study, two different leaf explants of water hayssop were cultured on 0.20 mg/l Thidiazuron (TDZ) containing Murashige and Skoog (MS) medium and exposed to White LEDs (W-LEDs), Blue LEDs (B-LEDs) or Red LEDs (R-LEDs) for inducing multiple adventitous shoots. Both explants induced multiple shoot buds after 6 weeks of culture but generated shoots when transferred to MS medium devoid of TDZ. Both explants responded in similar fashion as insignificant results were recorded for shoot formation frequency (%), shoot counts and shoot length. LEDs light significantlay affected the shoot counts and shoot length and highset shoot counts (27.73) and shoot length (1.59 cm) were recorded under W-LEDs. Interaction of both factors (explants × LEDs) only affected the shoot counts significantly and both explants generated maximum shoot counts under different LEDs. Upper half leaf (UHL) explants produced maximum shoot counts of 26.44 in response to B-LEDs. Whereas, Lower half leaf (LHL) explant prodcued 35.05 shoots under W-LEDs. In vitro regenerated shoots were rooted and acclimatized in water successfully.
Keywords: Explant, regeneration, in vitro, exogenous environment, LED light, TDZ

INTRODUCTION

Water hyssop (*Bacopa monnieri*) with a local name of Brahmi (India) belongs to Bacopa genus and Scrophulariaceae family. Genus Bacopa has over 100 species (Russo and Borreli, 2005) which shows wide distribution as wild or cultivated plant of wetlands or marshy areas (Behera *et al.*, 2016). It is a small semi-aquatic succulent, 10-30 cm long creeping herb with simple leaf and whitish or blueish flowers (Jain *et al.*, 2016). *B. monnieri* is known to be in use for centuries as complementary and alternative medicines (CAM) as a nerve tonic (Kean *et al.*, 2017). Other uses mentioned in "Charaka Samhita", an ancient Ayurvedic treatises include cognition, anxiety, diuretic, and heart and nervous system enegizer. It contains important bioactive compounds with Bacosides as major componnet used in modern era (Sivaramakrishna *et al.*, 2005).

In vitro shoot regeneration is dependant on variable factors ranges from plant to culture conditions and exogenous environment (Li *et al.*, 2011; Al-Tanbouz and Abu-Qauod, 2016). Among provision of exogenous environment, lighting source is an important factor which regulates the *in vitro* shoot induction and plant growth (Bello-Bello *et al.*, 2015; Sotthikul *et al.*, 2017). The light in the culture room or growth chambers are normally equipped with flourescent lights at variable light intensity and photoperiod. In recent years, LEDs are replacing the fluorescent lamps due to advantages like longer life, no heat emission with positive impact on plant growth and development (Bello-Bello *et al.*, 2015; Sotthikul *et al.*, 2017) alongwith its use for inducing *in vitro* shoot induction and secondary metabolites production (Schijlen *et al.*, 2006; Dorais *et al.*, 2008; Gangadhar *et al.*, 2013; Ouzounis *et al.*, 2015) of economic plant species. The most common used LEDs for *in vitro* shoot induction are White (W), Blue (B) or Red (R) or combinations of R:B in different concentrations or ratios (Karataş *et al.*, 2016, 2018).

Explant is another important factor that regulates the *in vitro* shoot induction which lead to axillary/adventitious shoots formation based on the presence or absence of meristematic cells. Leaf is an important but recalcitrant explant for most of the plant species but used suuceesfully for *in vitro* shoot formation of water hyssop. Full leaf explant of water hyssop is one of the most favourite explant for researchers and they used it successfully for inducing multiple shoots by offering variable culture conditions and plant growth regulators (Haque *et al.*, 2017; Mehta, 2017; Srivastava *et al.*, 2017; Ranjan *et al.*, 2018; Zote *et al.*, 2018). Whereas, Karatas *et al.* (2016) splitted the leaf explants in two parts (upper half and lower half) and regenerated adventitous shoots successfully. In this study, the potential of two different explants (upper half and lower half) of water hyssop under different LEDs lights were investigated.

MATERIALS AND METHODS

The leaves were taken from stock material (*in vitro* propagated and rooted plants) present in laboratory of Necmettin Erbakan University, Faculty of Science, Department of Biotechnology. As plant material was already taken from *in vitro* conditions, they were used directly for gaining two different explants; upper half leaf (UHL) and lower half part of leaf (LHL) by cutting the leaf from the middle in laminer flow cabin (Karataş *et al.,* 2016). Thereafter, boh explants were inoculated on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) enriched with 0.20 mg/l TDZ for 6 weeks followed by transfer to MS medium without TDZ for next 6 weeks. Data regarding shoot formation frequency, shoot counts and shoot length were taken after 12 weeks of culture. *In vitro* regenerated shoots were rooted according to Karatas and Aasim (2014) and acclimatized in water (Karatas *et al.,* 2013).

The MS medium used for regeneration and rooting was prepared by using MS (4.4 g/L), tea sugar (30 g/L) and agar (6.5 g/L). The pH of the medium was approximately autonmated at 5.8 with the aid of 1N HCl/NaOH after adding the TDZ. The identical culture conditions were given to both *in vitro* shoot formation and rooting by placing the culture plates (vitro shoot formation) or Magenta vessels (rooting). The growth room was maintained at 24±2°C, and light photoperiod (16/8 h: Light/darkness) was aided with W-LEDs, R-LEDS and B-LEDs.

The experimental set up was two factors (explants and LEDs) with three replicates containg 8 explants per replicate. The data taken and tabulated to Statistical Analysis using analysis of variance (ANOVA) with the help of SPSS 20.00 for Windows (SPSS Inc. Chicago, IL, USA). The post hoc tests were performed using Duncan's multiple range test DMRT) at p≥0.05 in order to compare the differences among treatments. The data were transformed to arcsine square root transformation (Snedecor and Cochran, 1967) before statistical analysis.

RESULTS AND DISCUSSION

Water hyssop is not a cultivated plant in India and its wild collection make this plant as one of the potential endangered species in future (Tiwari and Singh, 2010).Therefore, reserachers are working continuously for developing new *in vitro* regeneration protocols for its conservation and to meet its demand. The growth rooms or growth chambers used in these protocols were generally equipped with day light or fluorescent lamps. Recently, LEDs were used for inducing multiple shoots using leaf based explants (Karatas *et al.,* 2016) or shoot ip explants (Karatas *et al.,* 2018). Similarly, successful application of LEDs have been documneted for *in vitro* propagation of other important economic plants (Lian *et al.,* 2002; Baque *et al.,* 2011). Besides that, plant metabolites can be altered and several reports provide evidence of altering secondary metabolites in different plants due to LEDs. The present study describes the successful use of two different explants exposed to different LEDs lighting under *in vitro* conditions.

In vitro shoot multiplication is achieved through manipulating the different factors like explant or lighting source. For Bacopa, couple of studies revealed the efficient use of different explants like full leaf, UHL or LHL (Karatas *et al.*, 2016) or shoot tip explant (Karatas *et al.*, 2018) under different LEDs using BA (Benzylaminopurine) as cytokinin. In this study, upper and lower leaf half explants of water hyssop subjected to different LEDs lighting system induced multiple shoots on medium enriched with TDZ in almost similar fashion. Both explants led to induce shoot buds from leaf margins (cut end) followed by continous multiplication of green shoot buds but without sprouting of these buds into shoots until cultured on TDZ medium for 6 weeks due to suppressive efefcts of TDZ on shoot initiation (Karatas and Aasim, 2014). Contrarily, Karatas *et al.* (2016) reported direct shoot formation from margins of explants in response to BA under different LEDs. However, once the explants having multiple shoot buds were transferred to MS medium without

Table 1. Efficacy of explants and LEDs on *in vitro* shoot regeneration of water hyssop (B. *monnieri* L.).

	Treatment	**Regenration Frequency (%)**	**Shoots per explant**	**Shoot length (cm)**
Explant	Upper half leaf (UHL)	87.50^{ns}	22.28^{ns}	1.40^{ns}
	Lower half leaf (LHL)	84.72	23.31	1.39
LEDs	Red (R)	91.67^{ns}	17.59^{b}	1.19b
	White (W)	83.33	27.73^{a}	1.59a
	Blue (B)	83.33	23.06^{ab}	1.44ab
Explants × LEDs	U x R	95.83^{ns}	20.00^{b}	1.23^{ns}
	U x W	83.33	20.40^{b}	1.54
	U x B	83.33	26.44^{ab}	1.43
	A x R	87.50	15.19^{b}	1.15
	A x W	83.33	35.05^{a}	1.58
	A x B	83.33	19.68^{b}	1.45

**=significant (P<0.01) using DMRT, ns=non-significant

TDZ, these shoot buds sprouted and well developed shoots were recorded after 1 week of culture (Karataş and Aasim, 2014) but awaited for 6 weeks on MSO. Data regarding shoot formation frequency (%), shoot counts and shoot length were recorded after 12 weeks of total culture.

Comparasion of explants revealed the similar efficacy on shoot formation frequency (%), shoot counts and shoot length which were statistically insignificant. Shoot formation frequency (%) was recorded 87.50% for UHL and 84.72% for LHL. Previously, Karatas *et al.* (2016) reported 100% shoot formation frequency from these both explants in response to BA. Similarly, 100% shoot formation from leaf explant in response to TDZ has been highlighted by Karataş and Aasim (2014). Shoot counts and shoot length for UHL were documneted as 22.28 and 1.40 cm respectively. Whereas, shoot counts and shoot length for LHL was tabulated as 23.31 and 1.39 cm respectively. These results are similar to Karatas *et al.* (2016), who also documented the insignificant results for shoot counts using different leaf explants. However, shoot length was statistically significant and they recorded shoot length range of 1.01-1.54 cm after shifting the explants to MS medium.

Lighting system in grwoth rooms or growth chambers have potential to regulate the shoot induction behaviour. LEDs offer specific wavelength for plant growth under *in vitro* conditions (Budiarto, 2010; Chung *et al.,* 2010). Application of different LEDs resulted in insignificant effects on shoot formation frequency (%) compared to shoot counts and shoot length which were highly significant ($p \geq 0.01$). Shoot formation frquency ranged 83.33-91.67% with highest under R-LEDs light. Karatas *et al.* (2016, 2018) attained 100% shoot formation frequency under LEDs using BA in the culture medium. Least shoot counts (17.59) and shoot length (1.19 cm) were documneted under R-LEDs. On the other hand, similar shoot formation frequency of 83.33% was documented under both B-LEDs and W-LEDs. W-LEDs was best among all LEDs tested with highest shoot counts (27.73) and shoot length (1.59 cm). Karatas *et al.* (2016) also documneted the advatage of W-LEDs over different combinations of R:B- LEDs for generating highest shoot counts using different leaf explants. In another study by Karatas *et al.* (2018) revealed the W-LEDs least effective for maximum shoot counts compared to R:B LEDs combinations using BA and shoot tip explant. The difference in these studies is mainly due to difference in explant type, LEDs type and plant growth regulators. Results further highlighted the W-LEDs as least effetcive for generating longer shoots in their studies. There are some studies which also highlighted the better results under *in vitro* conditions using B or R LEDs (Chang *et al.,* 2003; Huan and Tanaka, 2004; Baque *et al.,* 2011).

Combination of light and cytokinins regulates the plant growth, physiological processes and *in vitro* regeneration potential. Comparative analysis of explant × LEDs induced statistically insignificant shoot formation frequency (%) and shoot length that ranged 83.33-95.83% and 1.15-1.58 cm, respectively. It was also recorded that both explants yielded highest shoot formation frequency and least shoot length under R-LEDs. Whereas, shoot length of both explants was highest under W-LEDs that was recorded as 1.54 cm for UHL × W-LEDs and 1.5 cm for LHL × W-LEDs. These results are contrarily to the previous findings of Karatas *et al*. (2016), who achieved longer shoots under R:B LEDs compared to W-LEDs. Comparaision of these results suggests that shoot length needs specific wavelength (Lian *et al.*, 2002; Li *et al.*, 2011) which in turn increase the net photosynthetic rate (Goins *et al.*, 1997). Lian *et al.* (2002) reported the triggering of photomorphogenic pigments which regulates the photoreception and regeneration.

On the other hand, response of both explants for highest shoot counts varied under different LEDs. Highest shoot counts of 35.05 were documneted under LHL × W-LEDs that was followed by 26.44 shoot counts in response to UHL × B-LEDs. In general, combination of explant × LEDs rvealed the need of specific wavelength for *in vitro* regeneration behaviour. Earlier study by Karatas *et al.* (2016) revealed the highest shoot counts from all leaf explants under W-LEDs. The results in this study are quite variable as different cytokinin was used in this study. It is supposed that LEDs may enhance the endogenous cytokinin production (Stirk *et al.*, 2011) which varied with type of LEDs and explant type (Rocha *et al.*, 2010).

This study highlights the successful use of two different explants for shoot induction by exposing explants to different LEDs with specific wavelength. The results suggest that both explants needs different LEDs for inducing highest shoot counts and shoot length is also dependant on specific wavelength generated by LEDs. This protocol can be used for inducing multiple shoots of Bacoap under *in vitro* conditions with the aid of LEDs.

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